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Exposure-Response Relationships and Drug Interactions of Sirolimus

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ABSTRACT

Sirolimus (rapamycin, RAPAMUNE, RAPA) is an immunosuppressive agent used for the prophylaxis of renal allograft rejection and exhibits an immunosuppressive mechanism that is distinct from that for cyclosporine and tacrolimus. The purpose of this manuscript is to discuss the exposure-response relationships and drug interactions of sirolimus. The various factors affecting sirolimus whole blood exposure included first-pass extraction, formulation, food, demographics, liver disease, assay method, and interacting drugs. Clinically significant effects caused by food, pediatric age, hepatic impairment, and interacting drugs require recommendations for the safe and efficacious use of sirolimus in renal allograft patients. An exposure-response model based on multivariate logistic regression was developed using the interstudy data from 1832 renal allograft patients. The analysis revealed an increased probability of acute rejection for sirolimus troughs <5 ng/mL, cyclosporine troughs <150 ng/mL, human leukocyte antigen (HLA) mismatches ≥4, and females. The outcomes suggested that individualization of sirolimus doses immediately after transplantation, based on HLA mismatch and sex, would likely decrease the probability of acute rejections in renal allograft recipients who receive concomitant sirolimus, cyclosporine (full-dose), and corticosteroid therapy. Sirolimus is a substrate for both Cytochrome P450 3A (CYP3A) and P-glycoprotein (P-gp) and undergoes extensive first-pass extraction. Drugs that are known to inhibit or induce these proteins may potentially affect sirolimus whole blood exposure. In healthy volunteers, cyclosporine, diltiazem, erythromycin, ketoconazole, and verapamil significantly increased sirolimus whole blood exposure, and rifampin significantly decreased sirolimus exposure. However, sirolimus whole blood exposure was not affected by acyclovir, atorvastatin, digoxin, ethinyl estradiol/norgestrel, glyburide, nifedipine, or tacrolimus. Among the 15 drugs studied, sirolimus significantly increased the exposures of only erythromycin and S-(-)verapamil.

KEYWORDS: sirolimus, exposure-response relationship, drug interactions.

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INTRODUCTION

Sirolimus (rapamycin, RAPAMUNE, RAPA) is an immunosuppressive agent used for the prophylaxis of renal allograft rejection.[1] The drug was isolated in a soil sample from Rapa Nui, an island in the South Pacific, hence the prefix "Rapa."[2] Sirolimus has a molecular weight of 914.2 g/mol.[3]

Sirolimus is neither a calcineurin inhibitor (as are cyclosporine and tacrolimus)[4] nor an antimetabolite (as are mycophenolate mofetil and azathioprine).[5] It has a unique cellular target called the mammalian target of rapamycin, or mTOR.[6] A protein kinase, mTOR is critical for cell cycle progression and cell proliferation. Sirolimus blocks mTOR, and this action inhibits cytokine-mediated proliferation in T cells, B cells, and mesenchymal cells, including smooth muscle cells.[2] The inhibitory effect of sirolimus on mTOR is initiated by binding of sirolimus to FKBP12 (an immunophillin), followed by binding of the RAPA-FKBP12 complex to an mTOR dimer.[7] Binding to mTOR blocks the 3 critical pathways, which include activation of translation for specific messenger RNA coding of cell cycle proteins,[8] activation of cyclin-dependent kinases required for coordinated DNA synthesis,[9,10] and synthesis of specific ribosomal proteins required for cell cycle progression.[11]

The currently approved regimens for Rapamune in the United States include both fixed-dose administration and dosing by concentration control.[1] The initial regimen is a fixed oral dose of sirolimus in combination with cyclosporine. This regimen is recommended in patients as soon as possible after transplantation; initiation is by a 6-mg loading dose on day 1 followed by a 2-mg/day maintenance dose thereafter. During sirolimus and cyclosporine combination therapy, sirolimus is administered 4 hours after cyclosporine capsules, *United States Pharmacopeia (USP)* or oral solution, *USP* [MODIFIED]. Cyclosporine, *USP* [MODIFIED], formulations (eg, Neoral) have an increased bioavailability compared with Sandimmune formulations.

Administration of sirolimus by concentration control is recommended (1) during a sirolimus and cyclosporine combination regimen in pediatric and hepatic-impaired patients, after coadministration with inhibitors and inducers of cytochrome P450 3A and P-glycoprotein, and after marked changes in cyclosporine doses and (2) during a cyclosporine withdrawal regimen in patients with low to moderate immunological risk. The cyclosporine withdrawal regimen is not recommended in patients with Banff grade III acute rejection, vas-

Table 1. Factors Affecting the Whole Blood Exposure of Sirolimus*

Factor	Sub-Factor	Comparison	n	Effect	*P* value
First-pass extraction	Solution	Intravenous vs oral solution†	21 159	F = 14%	95% CI (10%-17%)
	Tablet	Oral solution vs tablet‡	23 23	F = 17%	-
Food	High-fat meal (OS)	Fed vs nonfed§	22 22	AUC ↑ 35% in Fed	.001
	High-fat meal (T)	Fed vs nonfed§	24 24	AUC ↑ 23% in Fed	.001
Demographics	Ethnicity	Healthy black vs healthy white†	126 408	CL/F ↑ 34% in HB	.001
	Sex	Healthy females vs healthy males‖	38 267	CL/F ↑ 20% in HF	.002
	Age	Pediatric vs healthy adults	7 25	CL/F ↑ 90% in P	.037
Liver disease	Hepatic impairment	Hepatic-impaired vs healthy adults#	18 18	CL/F ↓ 33% in HI	.004
Assay method	Immunoassay	Immunoassay (MEIA) vs HPLC/UV**	194 133	Value ↑10% by MEIA Value ↑27% by MEIA	$R^2 = 0.87$ $R^2 = 0.81$

*OS indicates oral solution; F, oral systemic availability; CI, confidence interval; T, tablet; AUC, area under the curve; HB, healthy black; CL/F, sirolimus oral-dose clearance; HF, healthy female; P, pediatric; HI, hepatic-impaired; MEIA, microparticle enzyme immunoassay; and HPLC, high-performance liquid chromatography.

†Nonlinear mixed effects modeling (NONMEM) population analysis.

‡Crossover-design study.

§Within-study comparison (solution, tablet).

‖ Two-stage population analysis of 16 studies.

¶Interstudy comparison.

#Subjects in the 2 treatment groups were matched for age, sex, weight, and smoking habit.

**Whole blood samples from renal transplant patients analyzed by both MEIA and HPLC/UV methods.

cular rejection, dialysis-dependency, serum creatinine >4.5 mg/dL, retransplants, multiorgan transplants, or high panel reactive antibodies (PRA); nor is it recommended in black patients.

The purpose of this manuscript is to discuss the exposure-response relationships and drug interactions of sirolimus.

Sirolimus Exposure-Response Relationships

Factors Affecting Sirolimus Whole Blood Exposure

A summary of the factors that affect sirolimus exposure is provided in Table 1, which includes first-pass extraction, formulation, food, demographics, liver disease, and assay method.

The oral systemic availability (F) of sirolimus is low due to first-pass extraction. Based on a population analysis using software for nonlinear mixed effects modeling (NONMEM) and data from 13 clinical phase 1 studies, the systemic availability (F) of sirolimus from oral solution was ~14%.[12] A systemic availability of ~17% was predicted for the tablet based on the 27% increase in area under the curve (AUC) obtained from a crossover design study comparing sirolimus oral solution and tablet formulations.[1] Nevertheless, the oral solution and tablet formulations were shown to be therapeutically equivalent when a daily 2-mg sirolimus dose was administered to renal allograft patients (total n = 477) in combination with cyclosporine and corticosteroids.[13]

A high-fat meal increased the sirolimus whole blood AUC in healthy subjects by 35% after administration of oral solution[14] and by 23% after administration of the tablet.[1] In order to minimize the variability of whole blood sirolimus trough concentrations in renal allograft patients, it is recommended that sirolimus oral solution and tablets be taken consistently with or without food.[1]

Demographic characteristics, as expressed in ethnicity, sex, age, and liver disease, affected sirolimus oral-dose clearance (CL/F). Thus, CL/F was increased by 34% in healthy black subjects based on a NONMEM population pharmacokinetic (PK) analysis of 24 phase 1 studies.[15] However, no statistically significant differences in average whole blood sirolimus trough concentrations between black and nonblack renal allograft patients during the first 6 months after transplantation have been observed during phase III clinical trials.[1] Table 1 shows that sirolimus CL/F was increased by 20% in healthy female subjects based on a 2-stage population

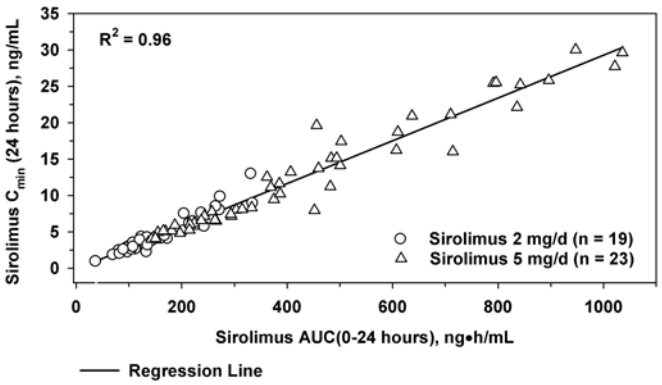

Figure 1. The trough-area relationship in renal allograft patients receiving concomitant doses of sirolimus, cyclosporine, and corticosteroids during a pivotal sirolimus phase 3 study.

PK analysis of 16 phase 1 studies (data on file at Wyeth), but dose adjustments in renal allograft patients based on gender are not recommended.[1]

An interstudy comparison of 7 pediatric dialysis patients (5-11 years) and 25 healthy adults (19-36 years) showed that CL/F was increased by 90% in the young pediatric patients.[16] Furthermore, liver disease affected the sirolimus PK; and in a population of Child-Pugh A (n = 13) and B (n = 5) hepatic-impaired patients, CL/F was decreased by 33%.[1] Based on these findings, it is recommended that whole blood sirolimus trough concentrations be monitored in pediatric patients and in patients with hepatic impairment.[1]

A prototype microparticle enzyme immunoassay (MEIA) was the primary assay for measuring sirolimus trough concentrations in whole blood samples from the phase 2 and 3 studies during the clinical development of sirolimus. This assay had a range of 3 to 30 ng/mL.[17] Based on an analysis of sirolimus concentration in blood samples from renal transplant patients by both MEIA and high-performance liquid chromatography HPLC with UV detection (HPLC/UV), the MEIA method showed a positive bias ranging from 10% (194 samples, 194 patients) to 27% (133 samples, 35 patients) compared with HPLC/UV in 2 clinical studies. The higher sirolimus concentrations by MEIA compared with HPLC/UV were due to the cross-reactivity of sirolimus metabolites.[18] Measurements of PK concentration-time profiles during phase 1, 2, and 3 studies were made by HPLC with tandem mass-spectrometric detection (HPLC/MS/MS) showing a range of 0.1 to 100 ng/mL.[19]

Pharmacokinetic Measures of Sirolimus Exposure in Phase 2 and 3 Clinical Trials

During the phase 2 and phase 3 development of sirolimus, trough concentrations (C_{min}) were measured in all patients, and 24-hour concentration-time profiles ($AUC_{0\text{-}24h}$) were measured in selected patients at some clinical sites.

Blood sampling over a dose interval at steady state permitted the determination of the trough versus AUC relationship. Figure 1 illustrates the relationship between $C_{min,24h}$ and $AUC_{0\text{-}24h}$ in 42 patients from a phase 3 pivotal trial of sirolimus versus azathioprine administered concomitantly with standard immunosuppressive therapy in renal allograft recipients. Patients received sirolimus doses of either 2 mg/d (shown by circles) or 5 mg/d (shown by triangles). Blood samples for PK profiling were generally obtained at 0, 1, 2, 3, 4, 5, 6, 8, 12, and 24 hours after dose administration for each patient at months 1, 3, and 6 after transplantation. The relationship was linear over a 30-fold range in trough concentrations, showing an $R^2 = 0.96$. The results in Figure 1 provided confidence that the more easily obtainable sirolimus trough samples were a useful surrogate for sirolimus AUC measurements.

Logistic Regression Analysis of Acute Rejection

Logistic regression analysis of acute rejection was studied using the drug concentrations and patient demographic/immunological characteristics from 4 pivotal phase 3 trials and 1 supportive phase 2 trial conducted during the clinical development of sirolimus.

The objective of this analysis was to evaluate the optimal dose of sirolimus in renal transplant patients who were at high risk and lower risk for acute rejection. The patients in the high-risk category were defined a priori as black patients, patients with PRA ≥ 50%, patients with mismatches of HLA ≥ 4, patients with retransplants, and patients with multiorgan transplants.

The following discussion includes sequential summaries of the (1) PK and statistical methods, (2) characterization of the clinical database, (3) exposure-response based on raw data and logistic regression modeling, (4) predicted probability analysis of statistically significant variables, and (5) concentration and dose predictions based on the final logistic regression and dose-proportionality models.

Pharmacokinetic and Statistical Methods

Based on the phase 2 and 3 protocol designs, sirolimus was to be administered as fixed doses. The maintenance dose (MD) was to be either 2 mg or 5 mg/d depending on the clinical study, and the loading dose was 3 × MD. However, sirolimus dose changes were allowed for toxicity at the investigator's discretion, and many of the doses were not fixed at 2 or 5 mg/d. Cyclosporine was administered by concentration control, and corticosteroids were administered according to a descending dose regimen.

Pharmacokinetic parameters were estimated using concentration and dose data up to 75 days after transplantation. Sirolimus and cyclosporine troughs were parameterized as

Table 2. Summary of Clinical Studies Used in Logistic Regression Analysis

Clinical Study	Sites Total/ United States*	Dosage Form	Maintenance Dose (mg/d)	Patients in Fixed-Dose RAPA Group†	Patients Analyzed
1	17/7	Oral Solution	2	97	95
2	40/40	Oral Solution	2, 5	558	468
3	34/6	Oral Solution	2, 5	446	424
4	30/22	Oral Solution Tablet	22	238239	175177
5	57/0	Tablet	2	525	493

*Clinical sites were located in Australia, Canada, Europe, and the United States.
†All patients received RAPA, Cyclosporine [CsA], and corticosteroids.

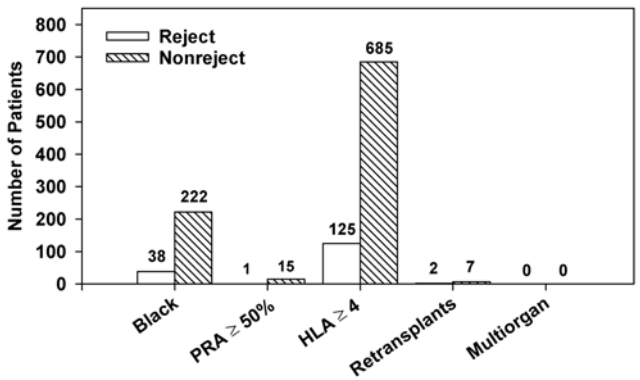

Figure 2. Distribution of high-risk renal allograft patients by rejections status among 5 clinical studies during the clinical development of sirolimus.

time-normalized (TN) values, which were estimated from the relationship $C_{min,TN} = AUC_{0\text{-}t}/t$, where $AUC_{0\text{-}t}$ is the area under the trough concentration-time curve to the time of first rejection (t). Dose-proportionality analysis used TN doses (ie, $Dose_{TN} = AUD_{0\text{-}t}/t$, where $AUD_{0\text{-}t}$ is the area under the dose-time curve) and a fit of $C_{min,TN}$ versus $Dose_{TN}$ was used to estimate α and ß from Equation 1:

$$C_{min,TN} = \alpha \cdot Dose_{TN}^{\beta} \quad (1)$$

The probability of acute rejection (pr) was determined from a general model for 1 of 2 possible outcomes using the SAS Logistic Procedure[20] as shown by Equation 2:

$$pr(Y=1) = \frac{1}{1+\exp\left[-\left(\beta_0 + \sum_{j-1}^{k} \beta_j X_j\right)\right]} \quad (2)$$

In patients with a biopsy-confirmed acute-rejection, *Y* was assigned a value of 1, and in patients without a biopsy-confirmed acute-rejection, *Y* was assigned a value of 0.

The parameter *k*, above the summation sign, is the number of independent variables.

The independent variables tested by logistic regression analysis fell into the categories of TN trough parameters (sirolimus, cyclosporine [CsA]), sex (female, male), race (black, nonblack), age (recipient, donor), donor type (cadaveric, living), PRA (≥50%, <50%), HLA mismatch (≥4, <4), and ischemia time.

Development of the final logistic regression model consisted of sequentially conducting univariate logistic regression as a preliminary test of each of the independent variables without correction for the other variables, followed by a stepwise multivariate logistic regression analysis. The criterion for the entry and removal of variables during the stepwise procedure was a *P* value < 0.15. The appropriate scale (linear versus nonlinear) was identified for significant continuous variables, and finally interaction-term testing was conducted on the preliminary final model.

Database Characterization

A summary of the clinical studies included in the logistic regression analysis is given in Table 2. Patient data were available from clinical sites in Australia (12), Canada (12), Europe (54), and the United States (53). The data show that (1) patients from United States sites were enrolled in all protocols except for study 5; (2) both solution and tablet formulations were used among the studies; (3) doses at 2 mg/d and 5 mg/d were used among the studies; and (4) not all patients in the fixed-dose treatments of the clinical studies were used in the analysis, which was due mainly to a lack of sirolimus or cyclosporine trough data in some patients.

Based on a patient breakdown by risk group (high risk versus lower risk) among the combined studies, there were nearly identical numbers of high-risk (n = 914) and lower-risk (n = 918) patients. All patients included in the analysis had demographic, PK, and acute rejection data, providing a homogeneous database. A higher rejection rate was observed for high-risk patients than for lower-risk patients in each of the 5 studies. Among all studies combined, the rejection rate was 14.9% in high-risk patients and 9.4% in lower-risk patients. These results illustrated the need for special consideration in treating high-risk patients.

The numbers of patients among each of the 5 high-risk categories by rejection status are shown in Figure 2. Only 2 of the

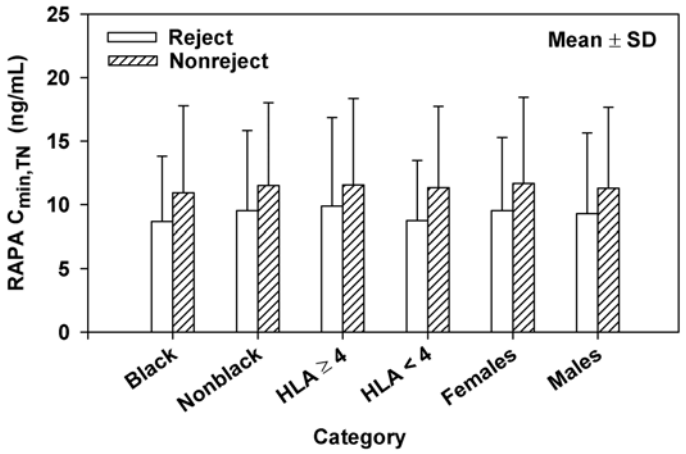

Figure 3. Sirolimus whole blood time-normalized trough concentrations ($C_{min,TN}$) by rejection status in high-risk and sex subcategories among 5 clinical studies during the clinical development of sirolimus.

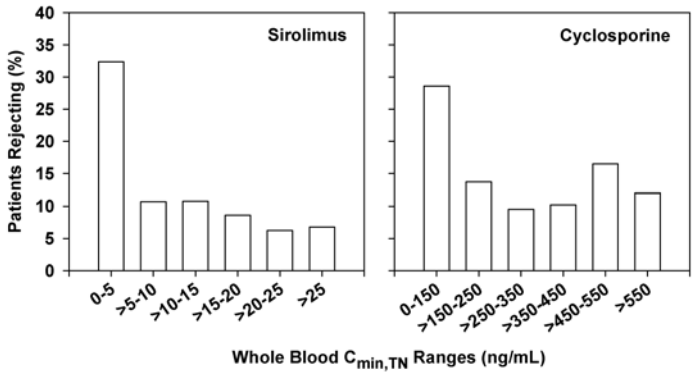

Figure 4. Acute rejection rates among sirolimus and cyclosporine whole blood time-normalized trough concentration ($C_{min,TN}$) subgroups in 5 clinical studies during the clinical development of sirolimus.

pre-assigned high-risk categories had sufficient data to be tested by logistic regression: black patients and HLA ≥ 4. There were too few subjects in the PRA ≥ 50% and retransplant categories for adequate testing, and there were no patients with multiorgan transplants, since all such patients were excluded from the clinical protocols.

A plot of sirolimus trough concentrations among the high-risk and sex subcategories by rejection status is shown in Figure 3. Nonrejecting patients showed higher mean sirolimus trough concentrations in all subcategories. However, intersubject variability was large in each of the subcategories, as reflected by the standard deviations; and a large overlap in concentrations was observed between subcategories. Obviously, these data would not permit sirolimus dose adjustments based on patient characteristics.

Exposure-Response Based on Raw Data and Logistic Regression Modeling

Drug concentration effects based on raw data are shown in Figure 4, which shows rejection rates by incremental concentrations ranges for whole blood sirolimus (left) and cyclosporine (right). The data for sirolimus suggest a sirolimus concentration-effect relationship, but the effect could be dichotomous because the percentage rejection is much higher at sirolimus troughs ≤5 ng/mL compared with troughs >5 ng/mL. The data for cyclosporine suggest only a dichotomous cyclosporine concentration-effect relationship because percentage rejection is much higher at cyclosporine troughs ≤150 ng/mL compared with >150 ng/mL, and the troughs >150 ng/mL appear to be randomly distributed over the remaining incremental groupings. Corresponding data for HLA mismatches showed a clear HLA mismatch-effect relationship, and percentage rejection increased smoothly as the number of HLA mismatches increased. Thus, HLA mismatches of 0, 1, 2, 3, 4, 5, and 6 yielded rejection percentages of 6.67%, 7.76%, 9.48%, 10.4%, 14.9%, 13.2%, and 20.4%, respectively.

Among the various independent variables tested by univariate logistic regression, only 4 variables showed *P* values suggesting that they may be useful predictors of acute rejection: sirolimus (*P* = .0001) and cyclosporine (*P* = .0600) TN troughs, sex (*P* = .0337), and HLA mismatch (*P* = .0001). It is noted that the *P* value for the high-risk category of race was not significant (*P* = .18), indicating that race would probably also not be significant in the multivariate analysis. However, the HLA mismatch variable included 65% of black patients (170/260); and thus race was not totally excluded from evaluation.

Prior to conducting multivariate logistic regression, testing was conducted for scale linearity and interaction terms. Testing for scale linearity showed that both the sirolimus and cyclosporine TN troughs were nonlinear by quartile breakdown and the Box-Tidwell transformation,[21] which indicated that the 2 drugs should be dichotomized in the final model. Therefore, sirolimus $C_{min,TN}$ values were dichotomized as either ≤5 or >5 ng/mL, and cyclosporine $C_{min,TN}$ values were dichotomized as either ≤150 or >150 ng/mL. The dichotomization limits for both drugs approximated the 10th percentile concentrations. The testing for interaction terms showed that there were no significant interactions among the independent variables.

Table 3 presents the results of the final multivariate logistic regression model for all variables dichotomized. The nonparenthetical values of the *P* values and odds ratios in Table 3 represent all variables dichotomized, and the parenthetical values represent sirolimus as a continuous variable. Sirolimus was analyzed as a continuous variable in order to be able to predict the dose adjustments needed for offsetting the effects of other independent variables. The magnitude of the odds ratios in the last column indicates a large effect

Table 3. Results From the Final Multivariate Logistic Regression Model

Independent Variable*	Wald Chi-Square *P* Value†	Odds Ratio Estimate†
Sirolimus (≤5 vs >5 ng/mL)	.0001 (0.001)	4.04 (0.94)
Cyclosporine (≤150 vs >150 ng/mL)	.0003 (0.0002)	2.66 (2.62)
HLA Mismatch (≥4 vs <4)	.0002 (0.0001)	1.74 (1.79)
Sex (female vs male)	.0175 (0.0260)	1.43 (1.39)

*HLA indicates human leukocyte antigen.

†Nonparenthetical values (all variables dichotomized); parenthetical values (sirolimus as a continuous variable).

on the probability of acute rejection below the dichotomization boundaries. For all variables dichotomized, the probability of acute rejection would be increased for sirolimus $C_{min,TN} \leq 5$ ng/mL (304%), cyclosporine $C_{min,TN} \leq 150$ ng/mL (166%), HLA mismatch ≥ 4 (74%), and females (43%). Based on the parenthetical values of the *P* values and odds ratio, the outcomes for cyclosporine, HLA, and sex changed very little when sirolimus was a continuous variable, which provided confidence in predicting dose adjustments for these characteristics.

Predicted Probability Analysis

The results of the multivariate logistic regression analysis in Table 3 provided an opportunity to determine the effect of cyclosporine concentrations, HLA mismatch, and sex on the probability of acute rejection over a range of continuous whole blood sirolimus concentrations.

Prediction of the probabilities of acute rejection associated with the various combinations of the significant independent variables was facilitated by Equations 3 and 4.

$$Pr = 1/(1 + e^{-\lambda}) \quad (3)$$

$$\lambda = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \beta_3 X_3 + \beta_4 X_4 \quad (4)$$

The probabilities (pr) were easily estimated at any sirolimus concentration using Equation 3 after calculating a value for λ from Equation 4. In Equation 4, β_0 is the intercept and β_1 through β_4 are the slopes from the final multivariate logistic regression model. Variables X_1 through X_4 are the independent variables for the model. Variable X_1 was a continuous variable for sirolimus $C_{min,TN}$. Variables X_2, X_3, and X_4 were dichotomized variables for cyclosporine, HLA mismatch, and sex, respectively, and were coded as either 1 (significant effect) or 0 (no significant effect).

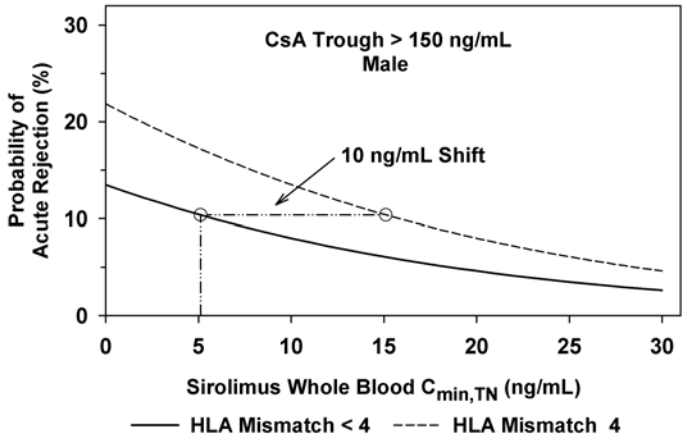

Figure 5. The predicted effect of HLA mismatch on the probability of acute rejection and sirolimus whole blood time-normalized trough ($C_{min,TN}$) requirements in male patients with CsA > 150 ng/mL.

Probabilities of acute rejection were estimated at sirolimus $C_{min,TN}$ values represented by the 10th, 50th, and 90th percentiles in both males and females based on the following combinations of CsA and HLA: CsA ≤ 150, HLA ≥ 4; CsA ≤ 150, HLA < 4; CsA > 150, HLA ≥ 4, and CsA > 150, HLA < 4. As expected, the lowest probability of rejection within each sirolimus percentile group occurred for the combination cyclosporine > 150 and HLA < 4; and the highest probability of rejection occurred for the combination cyclosporine ≤ 150 and HLA ≥ 4. The probability of rejection was higher for females than for males, and rejection probabilities decreased as sirolimus $C_{min,TN}$ values increased from the 10th through the 90th percentiles.

Sirolimus Concentration and Dose Predictions Based on Final Models

Estimation of the sirolimus doses needed to offset the increased probabilities of acute rejection (Table 3) was based on a 3-step approach. First, sirolimus troughs required to offset the increased probability of acute rejection were predicted. Second, dose proportionality of the data was determined. Third, sirolimus dose adjustments were predicted based on the results of the first 2 steps.

Predictions of sirolimus concentrations needed to offset the increased probabilities of acute rejection were based on the following rearrangement of Equation 2:

$$\lambda = -\text{Ln}\,[(1/Pr) - 1] \quad (5)$$

The estimation of λ from Equation 5 was based on a preselected probability of acute rejection referenced to the 10th percentile for sirolimus $C_{min,TN}$ values (or 5.1 ng/mL).

The resulting value of λ was then used in Equation 4, and the equation was solved for X_1 (sirolimus $C_{min,TN}$). Values of 1 or

Table 4. Designs Used in Sirolimus Drug Interaction Studies*

Regimen	Dosage Form	Sirolimus Dose (mg)	Studies (n)
SD	Oral solution	5, 10, 20	9
	Tablet	10, 15	2
MD	Oral solution	2, 4 (daily)	4

*SD indicates single dose; and MD, multiple dose.

0 were assigned to X_2, X_3, and X_4 to obtain contributions from the desired combination of independent variables. The results for one combination of variables are illustrated in Figure 5 (ie, male patient, CsA > 150 ng/mL, HLA ≥ 4, and HLA < 4).

The 2 curves in the figure were generated from the probability equation allowing sirolimus to vary from 0 to 30 ng/mL. The solid line represents patients with an HLA mismatch <4, and the dashed line represents patients with an HLA mismatch ≥4. The length of the horizontal dashed line reflects the increase in sirolimus concentrations needed to offset the increased probability of rejection. Figure 5 shows a 10 ng/mL shift in the curves for HLA mismatch ≥4, referenced to the probability of acute rejection at the 10th percentile for sirolimus $C_{min,TN}$. The final results of logistic regression analysis predicted that, to offset the increased probabilities of acute rejection for cyclosporine $C_{min,TN} \leq 150$ ng/mL, HLA mismatches ≥4, and female patients, sirolimus $C_{min,TN}$ needed to be 21.6, 15.1, and 10.8 ng/mL, respectively.

A dose proportionality analysis was conducted to assess the relationship between the sirolimus $C_{min,TN}$ and $Dose_{TN}$ using Equation 1. The resulting analysis yielded estimates for α = 5.196 and β = 0.686. The confidence interval (CI) for β (0.639 to 0.782) did not include the value of 1, which indicated that the relationship between $C_{min,TN}$ and $Dose_{TN}$ was not strictly dose proportional.

Therefore, a rearrangement of Equation 1 was used for predicting the required doses to offset the increased probability of acute rejection. Sirolimus $dose_{TN}$ values of 6.60, 3.92, and 2.4 mg/day were predicted for cyclosporine $C_{min,TN} \leq 150$ ng/mL, HLA mismatches ≥ 4, and female patients, respectively. In practice, only the HLA mismatch and sex characteristics would be useful for predicting the maintenance dose to be administered immediately after transplantation. Cyclosporine would not be included as there is no way to predict prior to transplantation which patients would show cyclosporine troughs <150 ng/mL.

Sirolimus Drug Interactions

First-pass extraction is the major determinant for sirolimus drug interactions. Sirolimus is a known substrate for hepatic[22] and intestinal[23] cytochrome P450 3A (or CYP3A), and 7 major metabolites of sirolimus have been identified in whole blood from healthy male subjects receiving a single oral dose

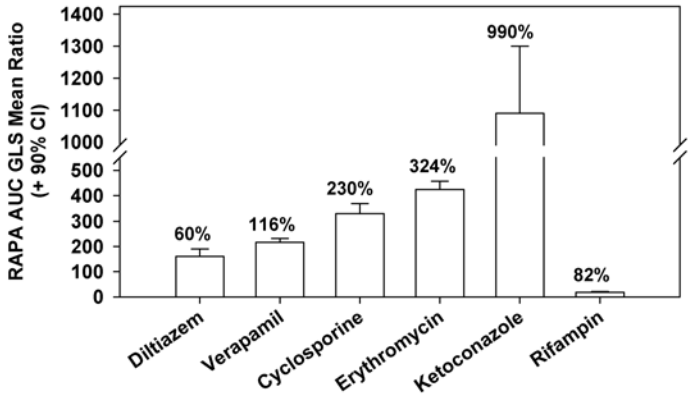

Figure 6. Coadministered drugs that significantly affected sirolimus whole blood AUC in healthy subjects. GLS indicates geometric least squares.

of sirolimus.[24] Sirolimus is also a known substrate for P-glycoprotein (P-gp).[25]

Prior to reaching the systemic blood after an oral dose, unchanged sirolimus must survive potential interactions with P-gp, an efflux pump located on the apical surfaces of enterocytes of the gut wall,[26] and CYP3A, a metabolic enzyme located within the interiors of enterocytes and hepatic cells.[27] Unchanged sirolimus can be recycled repeatedly by P-gp, which permits continued metabolism of the drug by CYP3A to maximize the intestinal first-pass extraction.[28] Drug interactions occur when any coadministered drug either inhibits or induces sirolimus efflux and/or metabolism.

Fifteen drug interaction studies have been conducted in healthy subjects during the clinical development of sirolimus. A brief summary of the study designs used in these studies is presented in Table 4. Most of the studies (11/15) were based on a single-dose regimen of sirolimus. All studies except 2 were conducted using the oral solution, and the oral doses of sirolimus varied depending on the regimen and interacting drug.

Five drugs significantly affected sirolimus whole blood exposure in healthy subjects as shown in Figure 6. The data for each drug include the geometric least squares mean ratio (+90% CI) of the AUC values for (sirolimus + interacting drug)/(sirolimus alone), together with the percentage increase in sirolimus AUC after coadministration with interacting drug. It can be seen that the effect of diltiazem[29] <verapamil <cyclosporine (Neoral capsules)[19] <erythromycin <ketoconazole,[30] all over a range of AUC increases from 60% to 990%. Rifampin decreased the sirolimus AUC by 82%.[1] In addition to the drugs shown in Figure 6, it has been reported that coadministration of sirolimus with the diazole antifungal agent voriconazole increased the sirolimus AUC by 11-fold.[1] Whole blood sirolimus concentration monitoring is recommended during the concomitant administration of sirolimus with either diltiazem, verapamil, or erythromycin. The coadministration of sirolimus with either ketoconzole, voriconazole, or

Table 5. Known Effects of the Drugs that Significantly Affected Sirolimus Exposure*

Drug	Effect on CYP3A	Effect on P-gp
Diltiazem	S,[34] I[35]	S,[36] I[37]
Verapamil	S,[38] I[39]	S,[40] I[41]
Cyclosporine	S,[42] I[43]	S,[44] I[43]
Erythromycin	S,[45] I[46]	S,[47] I[48]
Ketoconazole	I[49]	I[50]
Rifampin	Inducer[51]	Inducer[52]

*CYP3A indicates cytochrome P450 3A; P-gp, P-glycoprotein; S, substrate; and I, inhibitor.

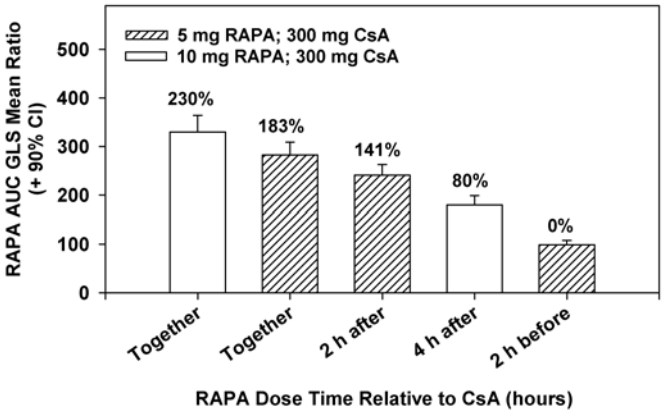

Figure 7. Effect of the relative sirolimus-cyclosporine dose times on sirolimus whole blood AUC in healthy subjects. GLS indicates geometric least squares.

rifampin is not recommended.[1] The known effects of the drugs in Figure 6 on CYP3Aand P-gp are listed in Table 5.

Each of the drugs that increased the sirolimus AUC is a substrate and inhibitor of CYP3A and P-gp, except for ketoconazole, which functions only as an inhibitor of CYP3Aand P-gp. Rifampin, which decreased sirolimus AUC, is an inducer of both CYP3A and P-gp.

Based on the data from 2 clinical studies in healthy subjects, it was possible to assess the effect of the relative sirolimus-cyclosporine dose times on whole blood sirolimus exposure. Figure 7 illustrates this effect in healthy subjects who received sirolimus simultaneously and 4 hours after cyclosporine in one study[19] and sirolimus simultaneously, 2 hours after, and 2 hours before cyclosporine in another study.[31] The results showed that the relative sirolimus-cyclosporine dose times had an effect on sirolimus exposure. Whole blood sirolimus exposure decreased in the following order of administration times: (1) sirolimus and cyclosporine given simultaneously produced either a 183% or 230% increase in sirolimus AUC, depending on the study; (2) sirolimus 2 hours after cyclosporine produced a 141% increase; (3) sirolimus 4 hours after cyclosporine produced an 80% increase; and (4) sirolimus 2 hours before cyclosporine produced no effect on whole blood sirolimus exposure.

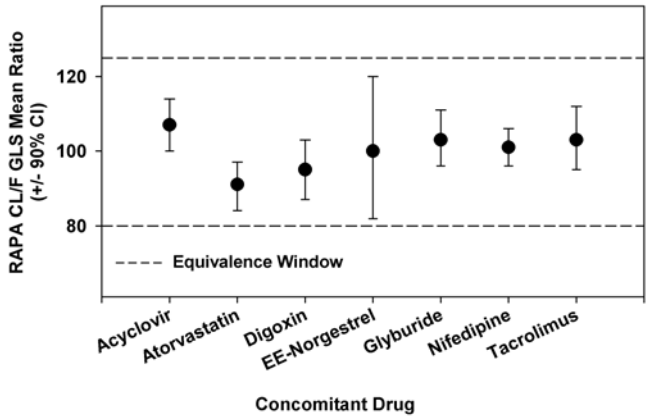

Figure 8. Coadministered drugs that did not significantly affect sirolimus whole blood CL/F in healthy subjects. GLS indicates geometric least squares.

Table 6. Known Effects of the Drugs That Did Not Significantly Affect Sirolimus Exposure*

Drug	Effect on CYP3A	Effect on P-gp
Acyclovir	-	-
Atorvastatin	S[53]	I[54]
Digoxin	-	S[55]
Ethinyl Estradiol	S[56]	-
Glyburide	-	-
Nifedipine	S,[57] I[35]	I[41]
Tacrolimus	S,[22] I[43]	S,[36] I[58]

*CYP3A indicates cytochrome P450 3A; P-gp, P-glycoprotein; S, substrate; and I, inhibitor.

Seven drugs that did not significantly affect sirolimus exposure in healthy subjects are shown in Figure 8. These drugs included acyclovir,[1] atorvastatin,[32] digoxin,[1] the ethinyl estradiol (EE)-norgestrel combination,[1] glyburide,[1] nifedipine,[1] and tacrolimus.[33] The 90% CIs for the geometric least squares mean ratios of sirolimus oral-dose clearance for each drug fell within the equivalence window of 80% to 125%. The known effects of the drugs shown in Figure 8 on CYP3A and P-gp are listed in Table 6.

In contrast to the drugs that increased the sirolimus AUC, the drugs in Table 6 functioned to a lesser degree as substrates and/or inhibitors of CYP3A[49]and P-gp[50]. Note, that only tacrolimus is both a substrate and inhibitor of CYP3A and P-gp. Two of the drugs, acyclovir and glyburide, were neither substrates nor inhibitors of CYP3A and P-gp.

Sirolimus did not affect the exposure of the vast majority of drugs listed in Tables 5 and 6 (ie, diltiazem, cyclosporine, ketoconazole, rifampin, acyclovir, atorvastatin, digoxin, EE, norgestrel, glyburide, nifedipine, and tacrolimus). However, sirolimus increased the AUC values of erythromycin by 69% and S-(–) verapamil by 48% (data on file at Wyeth Research).

CONCLUSION

The whole blood exposure of sirolimus was affected by first-pass extraction, formulation (oral solution vs tablet), age (pediatric vs adult), sex, ethnicity (black vs nonblack), liver disease (Child-Pugh A and B hepatic impairment), high-fat food, assay method (MEIA vs HPLC/UV), and interacting drugs (CYP3A inhibitor/inducers). The clinically significant effects due to food, pediatric age, hepatic impairment, and interacting drugs require recommendations for the safe and efficacious use of sirolimus in renal allograft patients.[1] Based on a multivariate logistic regression analysis, the probability of acute rejection in 1832 renal allograft patients was increased by sirolimus troughs <5 ng/mL, cyclosporine troughs <150 ng/mL, HLA mismatches ≥4, and female sex. Individualization of sirolimus doses immediately after transplantation, based on HLA mismatch and sex, would likely decrease the probability of acute rejection in renal allograft recipients receiving combined sirolimus, full-dose cyclosporine, and corticosteroid therapy.

Whole blood sirolimus exposure was increased in healthy subjects by coadministration with cyclosporine, diltiazem, verapamil, erythromycin, or ketoconazole. Rifampin significantly decreased sirolimus exposure in healthy subjects, and sirolimus significantly increased the exposures of erythromycin and S-(–) verapamil in healthy subjects.

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Appendix.

Clinical Investigators for Phase 2 and Phase 3 Rapamune Trials:

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Campbell (Princess Alexandra Hospital, Woolloongabba, Australia), V. Cambi (Universita Degli Studi, Parma, Italy), J. M. Campistol (Hopital Clinic I Provincial, Barcelona, Spain), M. Castagneto (Università Cattolica del Sacro Cuore, Rome, Italy), J. Chapman (Westmead Hospital, Westmead, Australia), K. Claesson (University Hospital, Uppsala, Sweden), E. H. Cole (The Toronto Hospital, Toronto, Ontario, Canada), D. J. Conti (Albany Medical College, Albany, NY), R. Cortesini (University of Rome Medical School, Rome, Italy), P. Daloze (Notre Dame Hospital, Montreal, Quebec, Canada), G. Danovitch (UCLA School of Medicine, Los Angeles, CA), P. Deteix (Hôpital Gabriel Montpied, Clermont-Ferrand, France), G. Duggin (Royal Prince Alfred Hospital, Sydney, Australia), J. F. Dunn (University of California at San Diego Medical Center, San Diego, CA), D. Durand (CHU Rangueil, Toulouse, France), J. Eris (Royal Prince Alfred Hospital, Camperdown, Australia), R. B. 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